U.S. Patent Application
Serial No. 09/322,353

Attorney Docket No. 9855-26U1
(OTT 3038-1)

Marked-Up Copy of Substitute Paragraphs, As Amended in the Amendment Corresponding to the Office Action Dated 30 January 2001

i) Please delete the paragraph at page 4, lines 2-3 and substitute in place thereof the paragraph amended to read as follows.

In a further aspect, the KDR^+ cells are isolated using a conjugated vascular ~~epithelial~~ endothelial growth factor or a molecule derived therefrom.

ii) Please delete the paragraph at page 14, lines 21-28 and substitute in place thereof the paragraph amended to read as follows.

The invention also includes a method of obtaining a cell population enriched for long-term repopulating human hematopoietic stem cells wherein KDR^+ cells are isolated using a conjugated vascular ~~epithelium~~ endothelial growth factor. This method simply capitalizes on the affinity of the KDR-VEGF receptor-ligand interaction to select cells expressing KDR on their surfaces by binding such cells, via the KDR present on the surface of the cell, to VEGF conjugated to, for example, a solid support matrix. Thus, the VEGF-conjugate can be used to affinity-purify the KDR expressing cells by standard methods well-known in the art.

iii) Please delete the paragraph at page 40, lines 16-18 and substitute in place thereof the paragraph amended to read as follows.

The mouse monoclonal antibody (clone 260.4), raised against the KDR soluble protein and recognizing the extracellular KDR domain, ~~were~~ was obtained from Gesellschaft für Biologische Forschung, GBF, Braunschweig, Germany.

iv) Please delete the paragraph at page 42, lines 1-14 and substitute in place thereof the paragraph amended to read as follows.

HPCs were seeded in 0.9% methylcellulose fetal calf serum free (FCS$^-$) medium supplemented with saturating amounts of HGFs [flt3, kit ligand (FL, KL), basic fibroblast GF (bFGF) (100 ng/ml each), interleukin 6 (10 ng), IL3 (100 U), granulomonocyte colony-stimulating factor (GM-CSF) (10 ng), G-CSF (500 U), M-CSF (250 U), thrombopoietin (Tpo) (100 ng), erythropoietin (Epo) (3 U)]. CFU-Mix/BFU-E and CFU-GM colonies comprised >5 x 103 and >10^3 cells, respectively (Gabbianelli et al., 1995, Blood 86:1661-1670). A more limited ~~HGFs~~ HGF combination comprised IL3, GM-CSF, Epo at the indicated dosages (Gabbianelli et al., 1995, Blood 86:1661-1670) (this culture condition was also utilized for NOD-SCID mice BM mononuclear cell (MC) clonogenic assay). CFU-Mix/BFU-E and CFU-GM colonies comprised >500 and >100 cells respectively. For detection of human colonies, the colony DNA was processed for PCR using KlenTaq-1 DNA polymerase (Clontech, Palo Alto, CA) and primers recognizing human a-satellite sequences on chromosome 17 (Warburton et al., 1991, Genomics 11:324-333).

24. (Amended) The method of claim 18, wherein the HPCs are isolated using an antibody that is specific for an early marker selected from the group consisting of CD34, Thy-1, c-kit receptor, flt3 receptor, AC133, vascular endothelial growth factor receptor I, vascular endothelial growth factor receptor III, Tie1, Tek, and basic fibroblast growth factor receptor.

26. (Amended) The method of claim 24, wherein the early marker is CD34.

39. (Amended) The method of claim 26, wherein HPCs are isolated by removing $CD34^+$ cells from the tissue using an antibody which specifically binds CD34 to yield $CD34^-$ HPCs.

40. (Amended) The method of claim 39, wherein the HPCs are isolated by removing lin^+ cells from the $CD34^-$ HPCs using an antibody which specifically binds a known lineage marker to yield $CD34^-lin^-$ HPCs .

41. (Amended) The method of claim 40, wherein the KDR^+ HSCs are isolated from the $CD34^-lin^-$ HPCs using an antibody which specifically binds KDR.

42. (Amended) The method of claim 41, wherein the antibody which specifically binds KDR is a polyclonal antibody.

43. (Amended) The method of claim 41, wherein the antibody which specifically binds KDR is a monoclonal antibody.

44. (Amended) The method of claim 43, wherein the monoclonal antibody is 260.4.

75. (New) The method of claim 18, wherein the HPCs are isolated using a method selected from the group consisting of isolating a cell based on a physical property of the cells, and isolating a cell based on a biochemical/biological property.

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Marked-Up Copy of Claims, as Amended in the Amendment Corresponding to the Office Action Dated 30 January 2001

1. (Amended) A method of obtaining a cell population enriched for long-term repopulating human hematopoietic stem cells (HSCs), ~~said~~ the method comprising ~~obtaining a population of cells from human hematopoietic tissue and isolating a population of KDR$^+$ cells therefrom,~~ isolating hematopoietic cells from a human hematopoietic tissue and separating cells that express KDR on their surface (KDR$^+$ cells) from cells that do not express KDR on their surface, thereby obtaining a KDR$^+$ cell population that is enriched for long-term repopulating ~~human hematopoietic stem cells~~HSCs.

2. (Amended) The method of claim 1, wherein ~~said human hematopoietic~~ the tissue is selected from the group consisting of ~~pre-embryonic hematopoietic tissue,~~an embryonic ~~hematopoietic~~ tissue, a fetal ~~hematopoietic~~ tissue, and a post-natal ~~hematopoietic~~ tissue.

deletion is new matter

3. (Amended) The method of claim ~~2~~ 1, wherein ~~said~~ the tissue is an embryonic ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of yolk sac, and ~~embryonic~~ liver.

4. (Amended) The method of claim ~~2~~ 1, wherein ~~said~~ the tissue is a fetal ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of ~~fetal~~ liver, ~~fetal~~ bone marrow, and ~~fetal~~ peripheral blood.

deletion is new matter

5. (Amended) The method of claim ~~2~~ 1, wherein ~~said~~ the tissue is a post-natal ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of cord blood, bone marrow, normal peripheral blood, mobilized peripheral blood, a hepatic ~~hematopoietic~~ tissue, and a splenic ~~hematopoietic~~ tissue.

deletion is new matter

6. (Amended) The method of claim 1, wherein ~~said~~ the KDR$^+$ cells are isolated using a reagent which specifically binds KDR.

7. (Amended) The method of claim 6, wherein ~~said~~ the reagent is an antibody ~~is selected from the group consisting of a polyclonal antibody and a monoclonal antibody~~.

8. (Amended) The method of claim 7, wherein ~~said~~ the antibody is a monoclonal antibody.

9. (Amended) The method of claim 8, wherein ~~said~~ the monoclonal antibody is 260.4.

10. (Amended) The method of claim 1, wherein ~~said~~ the KDR^+ cells are isolated using a conjugated vascular ~~epithelial~~ endothelial growth factor ~~or a molecule derived therefrom~~.

11. (Amended) The method of claim 1, wherein ~~said cells~~the HSCs are starvation resistant long-term repopulating human ~~hematopoietic stem cells~~HSCs.

18. (Amended) A method of ~~obtaining a purified population of~~ preparing long-term repopulating human ~~hematopoietic stem cells~~HSCs, ~~said~~ the method comprising ~~obtaining a population of cells from human hematopoietic tissue, isolating a population of hematopoietic progenitor cells therefrom, and isolating a population of KDR^+ cells from said population of hematopoietic progenitor cells, thereby obtaining a purified population of long-term repopulating human hematopoietic stem cells~~ isolating hematopoietic progenitor cells (HPCs) that express KDR on their surface (KDR^+ HPCs) from a human hematopoietic tissue and from HPCs that do not express KDR on their surface, whereby the isolated KDR^+ HPCs are long-term repopulating HSCs.

19. (Amended) The method of claim 18, wherein ~~said~~ the ~~human hematopoietic~~ tissue is selected from the group consisting of ~~pre-embryonic hematopoietic tissue,~~ an embryonic ~~hematopoietic~~ tissue, a fetal ~~hematopoietic~~ tissue, and a post-natal ~~hematopoietic~~ tissue.

20. (Amended) The method of claim ~~19~~ 18, wherein ~~said~~ the tissue is an embryonic ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of yolk sac, and ~~embryonic~~ liver.

delete as new matter?

21. (Amended) The method of claim ~~19~~ 18, wherein ~~said~~ the tissue is a ~~fetal~~ ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of ~~fetal~~ liver, ~~fetal~~ bone marrow, and ~~fetal~~ peripheral blood.

22. (Amended) The method of claim ~~19~~ 18, wherein ~~said~~ the tissue is a post-natal ~~hematopoietic~~ tissue ~~is~~ selected from the group consisting of cord blood, bone marrow, normal peripheral blood, mobilized peripheral blood, a hepatic ~~hematopoietic~~ tissue, and a splenic ~~hematopoietic~~ tissue.

23. (Amended) The method of claim 18, wherein ~~said hematopoietic progenitor cells are isolated using at least one~~ the KDR^+ HPCs are isolated from the tissue using a method selected from the group consisting of ~~isolation of cells expressing~~ isolating cells that express an early marker using ~~antibodies~~ an antibody specific for ~~said marker, isolation of cells not expressing~~ the early marker and isolating cells that do not express a late marker using ~~antibodies~~ an antibody specific for ~~said~~ the late marker, ~~isolation of cells based on a physical property of said cells, and isolation of cells based on a biochemical/biological property of said cells~~.

24. (Amended) The method of claim ~~23~~ 18, wherein ~~said early marker is~~ the HPCs are isolated using an antibody that is specific for an early marker selected from the group consisting of CD34, Thy-1, c-kit receptor, flt3 receptor, AC133, vascular endothelial growth factor receptor I, vascular endothelial growth factor receptor III, Tie1, Tek, and basic fibroblast growth factor receptor.

25. (Amended) The method of claim ~~23~~ 18, wherein ~~said late marker is~~ the HPCs are isolated using an antibody that is specific for ~~late marker is a~~ known lineage (lin) marker.

26. (Amended) The method of claim 24, wherein ~~said~~ the early marker is CD34.

27. (Amended) The method of claim ~~26~~ 18, wherein ~~said hematopoietic progenitor cells~~ the HPCs are ~~obtained~~ isolated from ~~said hematopoietic~~ the tissue using an antibody which specifically binds CD34 to select ~~a population of CD34$^+$ hematopoietic progenitor cells~~ CD34$^+$ HPCs.

28. (Amended) The method of claim 27, wherein ~~said population of KDR$^+$ cells is~~ the KDR$^+$ HPCs are isolated from ~~said~~ the ~~population of CD34$^+$ hematopoietic progenitor cells~~ CD34$^+$ HPCs using an antibody which specifically binds KDR.

29. (Amended) The method of claim 28, wherein ~~said~~ the antibody which specifically binds KDR is ~~selected from the group consisting of~~ a polyclonal antibody ~~and a monoclonal antibody~~.

30. (Amended) The method of claim ~~29~~ 28, wherein ~~said~~ the antibody which specifically binds KDR is a monoclonal antibody.

31. (Amended) The method of claim 30, whercin ~~said~~ the monoclonal antibody is 260.4.

32. (Amended) The method of claim 31, wherein ~~said cells~~ the KDR$^+$ HPCs are starvation resistant ~~human hematopoietic stem cells~~.

39. (Amended) The method of claim 26, wherein ~~said hematopoietic progenitor cells~~ HPCs are ~~obtained~~ isolated by removing CD34$^+$ cells from ~~said hematopoietic~~ the tissue using an antibody which specifically binds CD34 to yield CD34$^-$ HPCs~~select a population of CD34$^-$ cells~~.

40. (Amended) The method of claim 39, wherein ~~said hematopoietic progenitor cells are obtained~~ the HPCs are isolated by removing lin^+ cells from ~~said population of $CD34^-$ cells~~ the $CD34^-$ HPCs using an antibody which specifically binds ~~lin to~~ a known lineage marker to yield $CD34^-lin^-$ HPCs ~~select a population of $CD34^-lin^-$ cells~~.

41. (Amended) The method of claim 40, wherein ~~said population of KDR^+ cells is~~ the KDR^+ HSCs are isolated from ~~said population of $CD34^-lin^-$ cells~~ the $CD34^-lin^-$ HPCs using an antibody which specifically binds KDR.

42. (Amended) The method of claim 41, wherein ~~said~~ the antibody which specifically binds KDR is ~~selected from the group consisting of~~ a polyclonal antibody~~, and a monoclonal antibody.~~

43. (Amended) The method of claim ~~42~~ 41, wherein ~~said~~ the antibody which specifically binds KDR is a monoclonal antibody.

44. (Amended) The method of claim 43, wherein ~~said~~ the monoclonal antibody is 260.4.

51. (Amended) A method of expanding ~~a population of~~ long-term repopulating human ~~hematopoietic stem cells~~HSCs, the method comprising ~~obtaining a population of cells from human hematopoietic tissue, isolating a population of KDR^+ hematopoietic stem cells therefrom,~~ isolating HSCs that express KDR on their surface (KDR^+ HSCs) from a human hematopoietic tissue and incubating ~~said population of KDR^+ cells~~ the HSCs with vascular endothelial growth factor~~, thereby expanding said population of long-term repopulating human hematopoietic stem cells~~ to expand the HSCs.

52. (Amended) The method of claim 51, further comprising incubating ~~said~~ the population of ~~KDR^+ cells with at least one~~ HSCs with another growth factor.

53. (Amended) The method of claim 52, wherein ~~said~~ the other growth factor is selected from the group consisting of flt3 ~~receptor~~ ligand, kit ~~receptor~~ ligand, thrombopoietin, basic fibroblast growth factor, interleukin 6, interleukin 11, interleukin 3, granulomonocytic colony-stimulatory factor, granulocytic colony-stimulatory factor, monocytic colony-stimulatory factor, erythropoietin, angiopoietin, and hepatocyte growth factor.

69. (Amended) A method of isolating a ~~KDR+~~ stem cell capable of giving rise to at least one of a muscle cell, a hepatic oval cell, a bone cell, a cartilage cell, a fat cell, a tendon cell, and a marrow stroma cell, ~~said~~ the method comprising isolating a hematopoietic cell that expresses KDR on its surface from a human hematopoietic tissue, thereby isolating the stem cell. ~~KDR+ stem cell from hematopoietic tissue, thereby isolating a KDR+ stem cell giving rise to at least one of a muscle cell, a hepatic oval cell, a bone cell, a cartilage cell, a fat cell, a tendon cell, and a marrow stroma cell.~~

71. (New) The method of claim 69, wherein the tissue is selected from the group consisting of an embryonic tissue, a fetal tissue, and a post-natal tissue.

72. (New) The method of claim 69, wherein the tissue is an embryonic tissue selected from the group consisting of yolk sac and liver.

73. (New) The method of claim 69, wherein the tissue is a fetal tissue selected from the group consisting of liver, bone marrow, and peripheral blood.

74. (New) The method of claim 69, wherein the tissue is a post-natal tissue selected from the group consisting of cord blood, bone marrow, normal peripheral blood, mobilized peripheral blood, a hepatic tissue, and a splenic tissue.

75. (New) The method of claim 18, wherein the HPCs are isolated using a method selected from the group consisting of isolating a cell based on a physical property of the cells, and isolating a cell based on a biochemical/biological property.